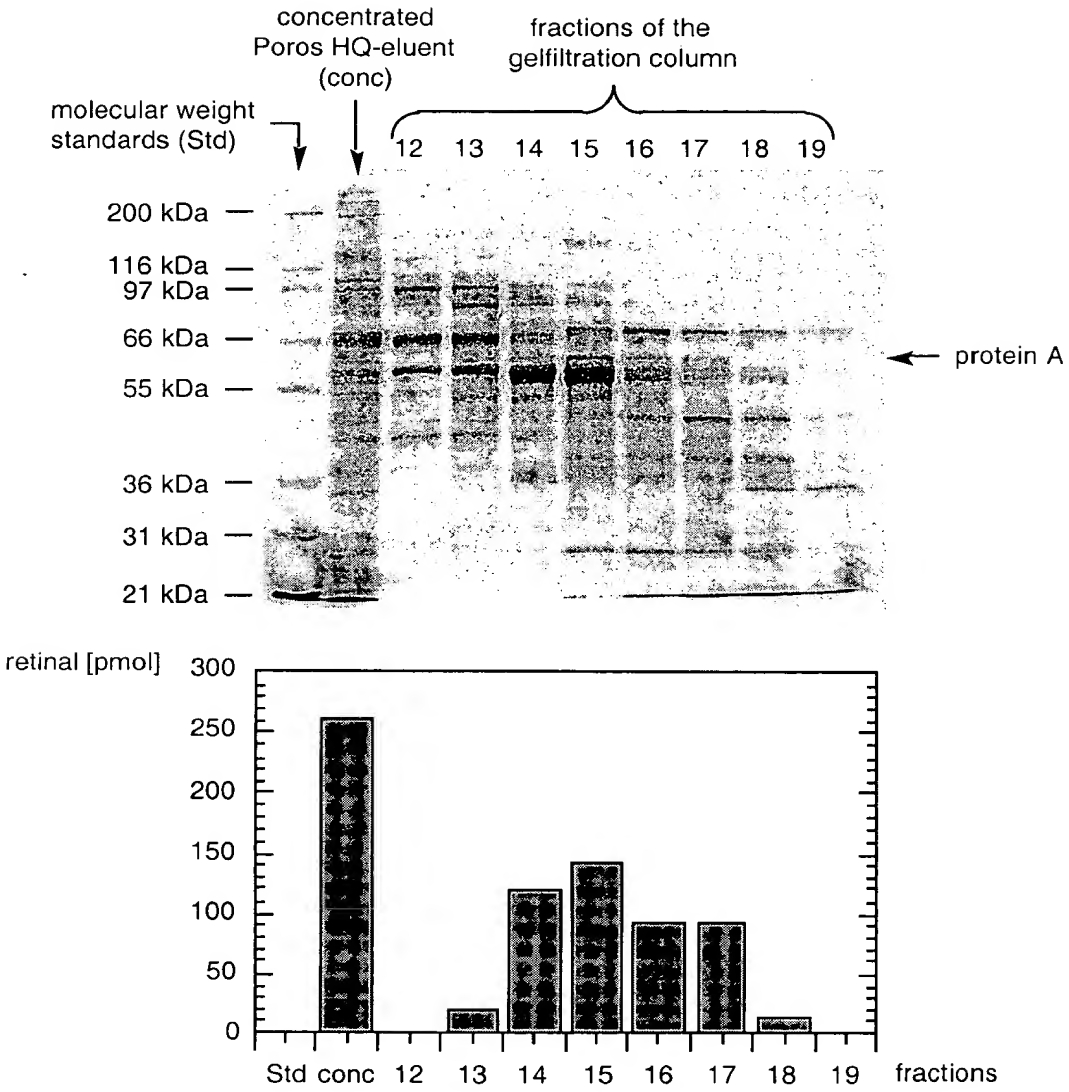
Figure 1


concentrated
Poros HQ-eluent
(conc)
fractions of the
gelfiltration column
molecular weight
standards (Std)
12 13 14 15 16 17 18 19
200 kDa
116 kDa
97 kDa
66 kDa
55 kDa
36 kDa
31 kDa
21 kDa
protein A
retinal [pmol]
300
250
200
150
100
50
0
Std conc 12 13 14 15 16 17 18 19
fractions

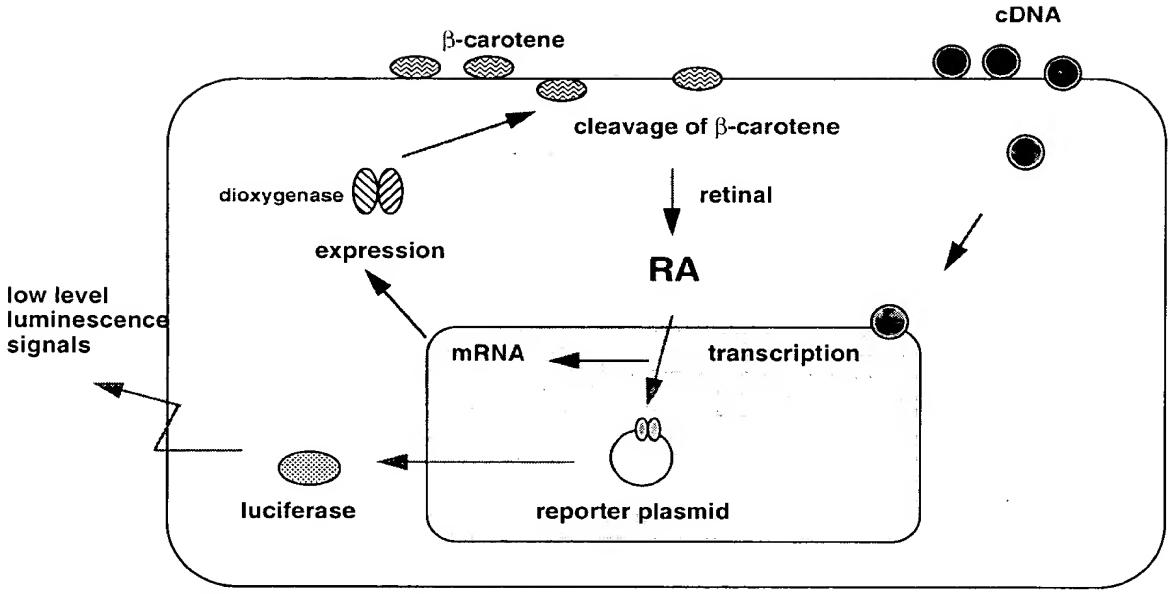

β-carotene
cDNA
cleavage of β-carotene
dioxygenase
retinal
expression
RA
low level
luminescence
signals
mRNA
transcription
luciferase
reporter plasmid

Figure 2

Figure 3 Seq. ID No. 2

```
       1  CGGATCCACT AGTAACGGCC GCCAGTGTGG TGGAATCCAT
CCTTCTATGT

      51  AACAGGAAAG AGCTGTTCTT AGCCCAGAGA GGAGGGCACC
GTACGCCTGC

     101  AGGAGCAGCT GGGTAGAGGA CACAGGAGAG CGATGGAGAC
AATATTTAAC

     151  AGAAACAAAG AAGAGCATCC AGAGCCCATA AAAGCTGAGG
TGCAAGGTCA

     201  GTTGCCCACT TGGTTGCAAG GGGTACTTCT CCGAAATGGC
CCAGGGATGC

     251  ACACAATAGG GGACACTAAA TACAACCACT GGTTTGATGG
CTTGGCTCTG

     301  CTGCACAGCT TCACGTTTAA AAATGGTGAA GTTTACTACA
GAAGTAAGTA

     351  CCTCCGAAGT GACACATACA ACTGCAATAT AGAAGCAAAC
CGAATCGTGG

     401  TGTCTGAGTT TGGAACCATG GCTTATCCGG ATCCATGCAA
AAACATATTT

     451  GCCAAGGCAT TCTCATACTT ATCTCACACC ATTCCTGAGT
TCACGGACAA

     501  CTGCCTGATC AACATTATGA AAACTGGGGA TGATTATTAT
GCTACCAGTG

     551  AGACTAACTT CATCAGAAAA ATTGATCCAC AGACTCTGGA
GACACTAGAT

     601  AAGGTAGACT ACAGCAAATA TGTAGCTGTA AACTTGGCAA
CTTCTCACCC

     651  ACACTATGAC AGTGCTGGAA ATATTCTCAA CATGGGTACT
TCAATTGTTG

     701  ATAAAGGGAG AACAAAATAT GTTCTCTTTA AGATCCCTTC
CTCTGTACCA

     751  GAAAAAGAAA AGAAGAAATC TTGTTTTAAA CACCTGGAAG
TAGTATGCTC

     801  CATCCCTTCT CGCTCCCTGC TCCAACCAAG CTACTACCAC
AGCTTTGGAA

     851  TCACAGAAAA TTATATTGTG TTCATAGAGC AGCCATTTAA
ACTGGATATT
```

901 GTCAAACTGG CAACTGCCTA CATCCGAGGT GTGAACTGGG CTTCCTGCCT

951 TTCCTTTCAT AAGGAGGATA AGACGTGGTT TCACTTTGTA GACAGAAAGA

1001 CGAAAAAAGA AGTATCCACC AAGTTTTACA CTGATGCTTT GGTGCTTTAT

1051 CACCACATAA ATGCTTACGA AGAAGATGGC CACGTTGTTT TTGATATCGT

1101 TGCCTACAGA GACAATAGCT TGTACGATAT GTTTTACTTA AAAAAACTGG

1151 ACAAAGACTT TGAAGTGAAC AACAAGCTTA CCTCCATCCC AACCTGCAAG

1201 CGCTTTGTTG TGCCTCTGCA GTATGACAAG GATGCAGAAG TAGGTTCTAA

1251 TTTAGTCAAA CTTCCAACTT CCGCAACTGC TGTAAAAGAA AAAGATGGCA

1301 GCATCTATTG TCAACCTGAA ATATTATGTG AAGGGATAGA ACTGCCTCGT

1351 GTCAACTATG ACTACAATGG CAAAAAATAC AAGTATGTCT ATGCAACAGA

1401 AGTCCAGTGG AGCCCAGTTC CTACAAAGAT TGCAAAACTG AATGTCCAAA

1451 CAAAGGAAGT ACTGCACTGG GGAGAAGACC ACTGCTGGCC CTCAGAGCCC

1501 ATCTTTGTTC CCAGCCCCGA TGCAAGAGAA GAGGATGAAG GTGTTGTTTT

1551 GACCTGTGTT GTGGTGTCTG AGCCAAATAA AGCACCCTTC CTACTCATCT

1601 TGGATGCTAA AACATTCAAA GAATTGGGCC GAGCCACAGT TAACGTAGAA

1651 ATGCATCTGG ACCTGCATGG GATGTTTATA CCACAGAATG ATTTGGGGGC

1701 TGAGACGGAA TAAAACGCTA TTGATCCGAC TACACAAACT GAGACAACTT

1751 TCTACTGAAC ATGAGTTAAT ATCCCTTTTA CCATTCAAGA ACAACCATAT

1801 AACGACACAA AATGACTATG TATAATCTCT TAAATAATAG ATATAATCCT

1851 TTTAAGGCAC AGCGATGAGT TTTACTACAG GTAACGATAT GCACAACTGG

1901 CATATAACTA TTCCAAAAGA AGAAGAACGA TCAGTGTTTT AGAAGTGCTA

1951 ATGTTGTACA TAACGGCGGC AGAGGGAACA GGAGAGAAAG GTAACGGGAA

2001 TATTTAATAG AATATAGATT TCTGAGCAAA TGAAGTGCAG TATTTATGGT

2051 GTGATGCATG GCATGAGTCA CATAGGTCTG CAGCTCATGT ATCTTTTAGA

2101 GATCGTTTCA AGATTGCAGC TTGTGATGCA AGTTTTCTCC AGCCAGAAAA

2151 CCTCATTTTA AACCATCTGC TACTGGTAAT TCATACCAAT GCATTTTCTT

2201 GGTGCTCGAT TTACACTATA ACCAAAGTTA AGTATTACAT TCAGGTGCTA

2251 CAACTTTCTA ATTTACAACC GAAACAAACA AGCAAACAGC ACTTGCTTTG

2301 CTAATAACCC CATGGTGTAT TTTTCCTTTT TATGATGACA AAACCAAGTA

2351 CATATGGTTT TATGTAGCAT TCAATTATAC TTCAGTGCTA TTCCATCCTA

2401 ATGTTATAAG CAATTTGTAT TTAAATCAGT TTTCCTTGAG AATATCTGAC

2451 ATAACATTTT GTGTAATGAG ATGACTATGT TGTCTAAAGA TGAACAGGAA

2501 TGTATCTTTT ATTAGTATTG TTAATTGTGT TACTAATACT ATGCATATGA

2551 ATGAGAGCAA TGTATTTCTA GGAGAACTCA GATATACATT CAACAATTTC

2601 TGTAGGTGAA AATGCATTTA CTGATGAAAG TTGAATCGTT AATGAGGGAG

2651 AAAACTGGGT ATCCATCCAT CCAACTATGT TAGGTGTTCA CCTGGTCTGT

2701 ATGTGACACC ACGCTGTTTG GGTATCTCTC ACTTTCACAT ACCTGTTCTC

2751 ATGGTTTCTG CTACTCACTG TATTTTGCAG GAGAGAAACA AAATGAAATC

2801 ACTGTCACTT ACTATCGCCC CATCACATAA GAACAATGGG GCTTTGGTGA

2851 CTTGTTCATG ATTACATAAG ATGTTTGCAG CAGAGCAGCA ATAGAACCAA

2901 CACCATCCAC AGTTCTTGCT TGCTCTGTTA TGACTCCCTT TGCTGTCTTT

2951 ATGGTTTGCA TGTATGAAGA ATACACTGCC TAATTCTAAT GTTAAAAAGT

3001 CACTGGGGTC AGATCTAGAG CTTAAGTAAG CAGTCTGGGG TTTTCAAATG

3051 TTTATATGTT CCATAAAATG GAAATAAACA CCTCCATAAT AAAAAAAAAA

3101 AAAAAAAAAA A

Figure 4 Seq. ID No. 1

```
       1  METIFNRNKE EHPEPIKAEV QGQLPTWLQG VLLRNGPGMH
TIGDTKYNHW

      51  FDGLALLHSF TFKNGEVYYR SKYLRSDTYN CNIEANRIVV
SEFGTMAYPD

     101  PCKNIFAKAF SYLSHTIPEF TDNCLINIMK TGDDYYATSE
TNFIRKIDPQ

     151  TLETLDKVDY SKYVAVNLAT SHPHYDSAGN ILNMGTSIVD
KGRTKYVLFK

     201  IPSSVPEKEK KKSCFKHLEV VCSIPSRSLL QPSYYHSFGI
TENYIVFIEQ

     251  PFKLDIVKLA TAYIRGVNWA SCLSFHKEDK TWFHFVDRKT
KKEVSTKFYT

     301  DALVLYHHIN AYEEDGHVVF DIVAYRDNSL YDMFYLKKLD
KDFEVNNKLT

     351  SIPTCKRFVV PLQYDKDAEV GSNLVKLPTS ATAVKEKDGS
IYCQPEILCE

     401  GIELPRVNYD YNGKKYKYVY ATEVQWSPVP TKIAKLNVQT
KEVLHWGEDH

     451  CWPSEPIFVP SPDAREEDEG VVLTCVVVSE PNKAPFLLIL
DAKTFKELGR

     501  ATVNVEMHLD LHGMFIPQND LGAETE
```

Figure 5 Seq ID No. 4 and Seq ID No. 5

```
        10 EEHPEPIKAEVQGQLPTWLQGVLLR..NGPGMHTIGDTKYNHWFDGLALL
57
           ||   |: | | |.:| || | |||   |||:  :|   : | ||| |||
        20 EELSSPLTAHVTGRIPLWLTGSLLRCFTGPGLFEVGSEPFYHLFDGQALL
69
        58 HSFTFKNGEVYYRSKYLRSDTYNCNIEANRIVVSEFG..TMAYPDPCKNI
105
           | | || | | |  :::|.| |   .   |||:.|||  | |:|||||||
        70 HKFDFKEGHVTYHRRFIRTDAYVRAMTEKRIVITEFGFTTCAFPDPCKNI
119
       106 FAKAFSYLSHTIPEFTDNCLINIMKTGDDYYATSETNFIRKIDPQTLETL
155
           |.: |||       | ||| |:|:   |:|||| .||||| ||.|:||||:
       120 FSRFFSYFRGV..EVTDNALVNVYPVGEDYYACTETNFITKINPETLETI
167
       156 ..DKVDYSKYVAVNLATSHPHYDSAGNILNMGTSIVDKGRTKYVLFKIPS
203
              .||   ||.|| ||.||| :. | : |.|          | : |||
       168 FTKQVDLCNYVSVNGATAHPHIENDGTVYNIGNCFGKNFSIAYNIVKIPP
217
       204 SVPEKEKKKSCFKHLEVVCSIPSRSLLQPSYYHSFGITENYIVFIEQPFK
253
              :||   | |   |:|   |     .||| ||||:| ||||||:| | |
       218 LQADKEDPISKFTS.EIVVQFPCSDRFKPSYVHSFGLTPNYIVFVETPVK
266
       254 LDIVKLATAY.IRGVNWASCL.SFHKEDK.TWFHFVDRKTKKEVSTKFYT
300
           :.: |  ..: : | |:  |  ||  |    | |  |:| || .. |: |
       267 INLFKFLSSWSLWGANYMDCFESFTNETMGVWLHIADKKRKKYLNNKYRT
316
       301 DALVLYHHINAYEEDGHVVFDIVAYRDNSL...YDMFYLKKLDKDFE...
344
           |:|||| ||:.| .: |:  ::       |   ||  | ...|
       317 SPFNLFHHINTYEDNGFLIVDLCCWKGFEFVYNYFTLYLANLRENWEEVK
366
       345 VNNKLTSIPTCKRFVVPLQYDKDAEVGSNLVKLP.TSATAV..KEKDGSI
391
           | :   |  :|:|.||  || |: | ||| || |.|||:   :.  .|
       367 KNARKAPQPEVRRYVLPLNIDK.ADTGKNLVTLPNTTATAILCSDEFTTI
415
       392 YCQPEILCEG....IELPRVNYD.YNGKKYKYVYATEVQWSPVPTKIAKL
436
           : :||:|  |     | |.:||  | || | | |   .    || :: ||
       416 WLEPEVLFSGPRQAFEFPQINYQKYCGKPYTYAYGLGLNHF.VPDRLCKL
464
```

```
437 NVQTKEVLH..WGEDHCWPSEPIFVPSPDAREEDEGVVLTCVVVSEPNKA
484
    ||.|||     | |   :|||||||  ||| |||:|||| . ||     .
465 NVKTKETWFTVWQEPDSYPSEPIFVSHPDALEEDDGVVLSVVVSPGAGQK
514
                .          .          .
485 P.FLLILDAKTFKELGRA..TVNVEMHLDLHGMF 515
    | :||||.||   |. ||  || : . .  ||:|
515 PAYLLILNAKDLSEVARAEFTVEINIPVTFHGLF 548
```

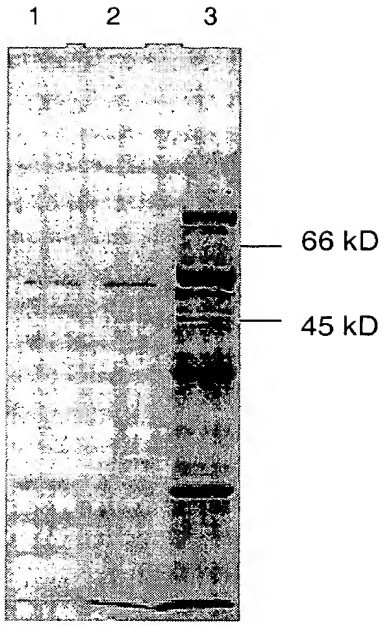


Fig. 6 shows a 10% polyacrylamide gel with E.coli expressed β,β-carotene 15,15'-dioxygenase after affinity tag purification; lane 1 and lane 2: 2 fractions from the Co^{2+}-chelate column showing the main band at 60 kD; lane 3: low range molecular weight marker (Bio Rad).

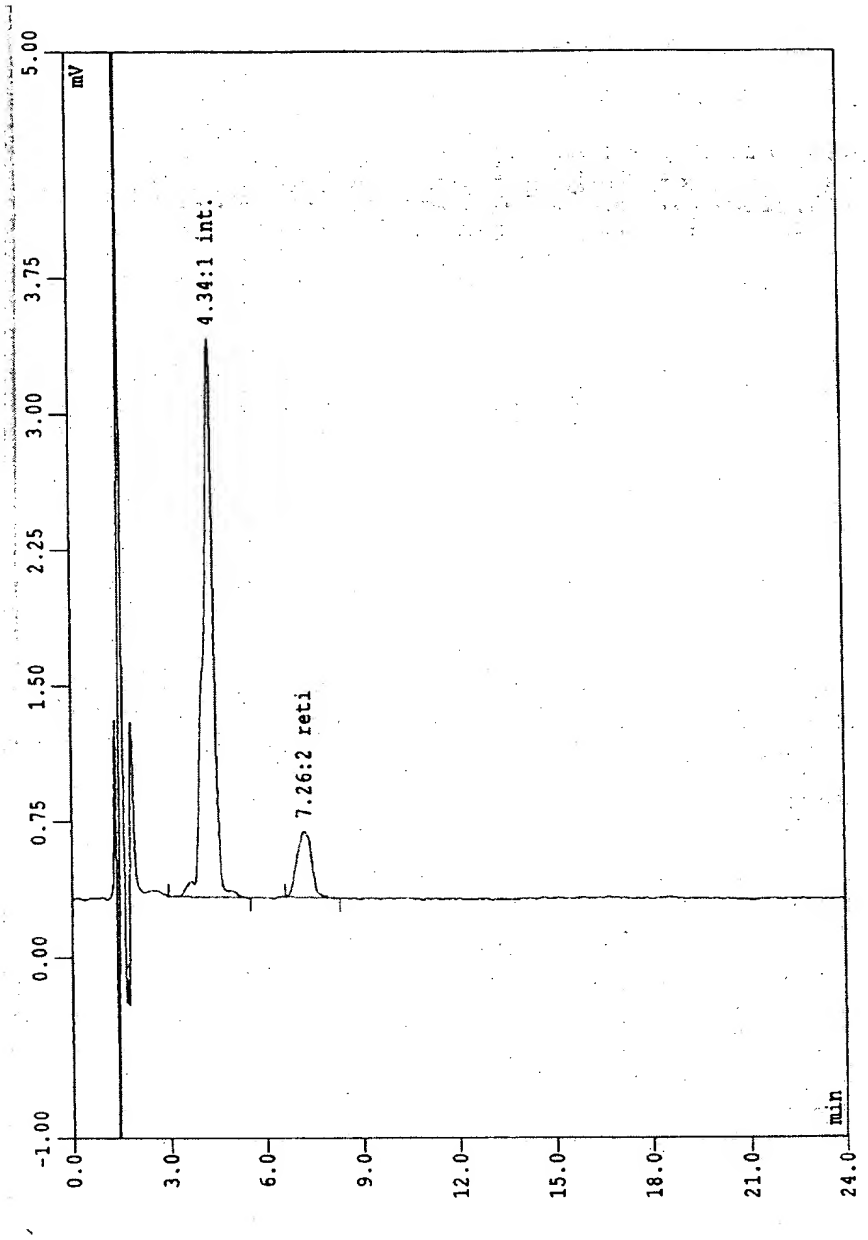


Fig. 7 shows an HPLC profile of the reaction mixture at the end of an actvity assay for the β,β-carotene 15,15'-dioxygenase following the procedure in example 1. The first peak in the chromatogram represents the internal standard, while the second peak corresponds to retinal as the only product formed during the central cleavage with β-carotene as substrate.

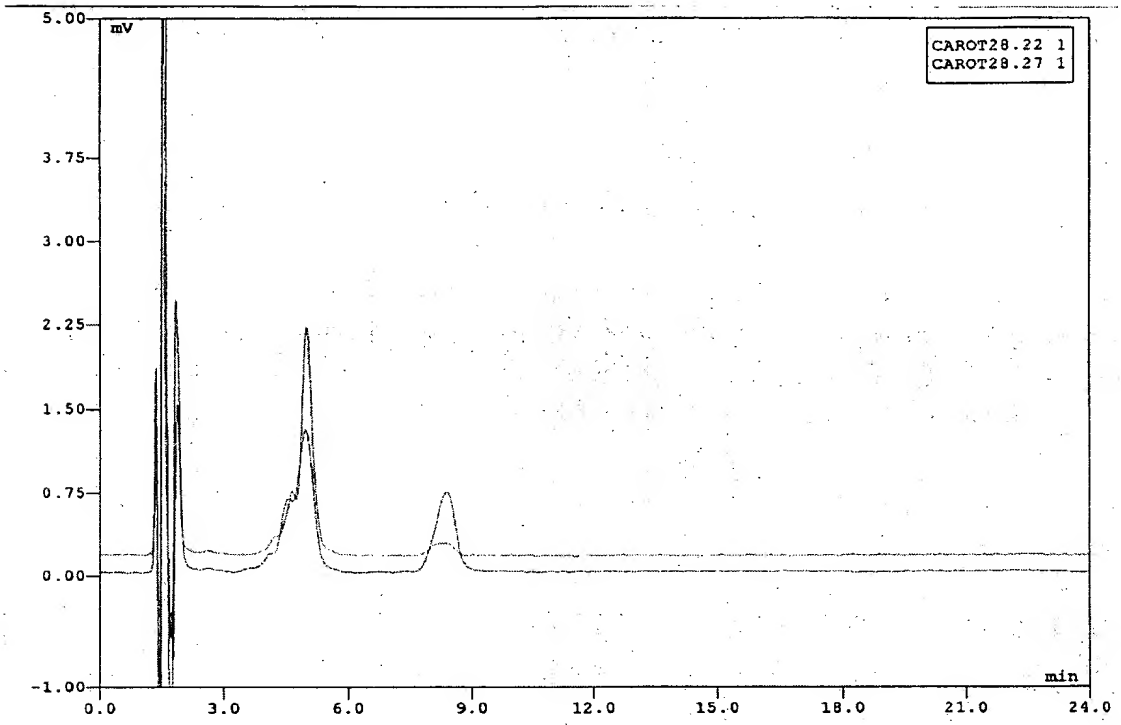


Fig. 8 confirms that the product peak in Fig. 7 is indeed retinal. A sample which was positive in the activity assay (green chromatogram) was spiked with retinal and analysed in second HPLC run (red chromatogram). The chromatograms of the two runs were then overlayed.